FBS40 – QIAsymphony SP DNA Extraction

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1. Scope

1.1. This procedure describes the extraction and isolation of deoxyribonucleic acid (DNA) from biological specimens recovered from evidentiary items for nuclear DNA typing using the QIAsymphony DNA Investigator Kit and QIAsymphony SP instrument.

2. Background

2.1. The QIAsymphony SP and the QIAsymphony DNA Investigator kit utilize magnetic-particle technology to perform fully automated purifications of nucleic acids. Samples are run in batches of 24 with a capacity of up to 96 samples. The lysis volumes for the Casework ADV HE protocols include 200 µL, 500 µL, and 1000 µL depending on the sample and its condition. Magnetic bead particles with a silica surface will bind the DNA and isolate it from lysates when in the presence of a chaotropic salt. Next, a magnetic rod separates the magnetic bead particles from the lysates. Finally, the DNA is washed and eluted in 40 µL Buffer ATE.

3. Safety

3.1. Wear personal protective equipment (e.g., lab coat, gloves, mask, eye protection), when carrying out standard operating procedures (SOPs).

3.2. Read Safety Data Sheets (SDSs) to determine the safety hazards for chemicals and reagents used in the SOPs.

3.3. Do not use bleach to clean or disinfect the QIAsymphony SP instrument. Toxic fumes can be produced when bleach comes into contact with salts from the buffers.

3.4. If liquid containing these buffers is spilt, clean with a suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 10% bleach followed by 70% ethanol.

4. Materials Required

4.1. QIAsymphony DNA Investigator Kit – Store at room temperature (15-25°C):

4.1.1. Reagent cartridge – Following first use, store for a maximum of 2 weeks. If partially used, replace the cover of the trough containing the magnetic particles, seal the buffer troughs with the provided Reuse Seal Strips, and close the carrier RNA tubes with screw caps. This should occur immediately following the end of the protocol run to avoid evaporation. The reagent cartridge should be open for a maximum of 15 hours.

4.1.2. Carrier RNA (cRNA) – Prior to the first use of the QIAsymphony DNA Investigator kit, dissolve the lyophilized cRNA in 1.6 mL Buffer ATE. Transfer 400 µL to each tube in positions one and two of the enzyme rack located on the reagent cartridge. Add an additional 1.2 mL Buffer ATE to each tube and mix by pipetting up and down several times.

NOTE: The final volume for the tubes containing cRNA must be exactly 1.6 mL. Store the reconstituted cRNA at -20°C. Do not freeze-thaw the reconstituted cRNA more than 3 times.

4.1.3. QIAGEN Proteinase K – Store at room temperature (15-25°C). To store for extended periods of time, store at 2-8°C.

4.1.4. Enzyme rack

4.1.5. Piercing lid

4.1.6. Buffer ATE (20 mL)

4.1.7. Buffer AVE (20 mL)

4.1.8. Buffer ATL

4.1.9. Reuse Seal Strips

4.2. Consumables:

4.2.1. 200 µL filter tips

4.2.2. 1500 µL filter tips

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4.2.5. Tip disposal bag

4.2.6. Sample tubes

4.2.7. Elution tubes

4.3. Additional Reagents:

4.3.1. Buffer ATL

4.3.2. QIAGEN Proteinase K

4.3.3. TopElute Fluid (60 mL)

4.3.4. 1.0 M DTT (FBR38)

4.4. Adapters:

4.4.1. Samples tube carrier

4.4.2. Secondary tube adapter insert 3b

4.4.3. Cooling adapter, 2 mL (P/N 9020674)

4.4.4. Non-cooling adapter, 2 mL (P/N 9021670)

5. Standards and Controls

5.1. At least one reagent blank (i.e., extraction control) must be prepared and processed in parallel with each batch of evidentiary specimens processed for DNA typing purposes. The reagent blank(s) (RB) is comprised of all the reagents used in the analytical process and is carried through the same extraction, quantification, amplification, and detection procedure(s) as the evidence samples. When running multiple batches at a time, at least one reagent blank must be included on each carrier.

5.2. For differentials, the reagent blank created in conjunction with the isolation of the epithelial cell fraction is designated as the non-sperm fraction (BK#.E in Sample Tracking and Control Solutions (STACS)). The reagent blank created in conjunction with the isolation of the male fraction is designated as the sperm fraction (BK#.S).

5.3. The RB will always be the last sample processed in a set. Any RBs created must match the volume of the most concentrated sample.

6. Procedures

6.1. Batch Preparation

6.1.1. Place each sample into a labeled, sterile tube. Large samples can be cut into smaller pieces to fit more conveniently.

6.1.2. Evaluate case scenario, sample source, substrate type, and/or serology results to determine the appropriate extraction protocol for each sample. Then determine which samples can be grouped and processed as a batch.

6.1.3. Samples suspected of containing low levels of DNA should be extracted in separate batches from samples suspected of containing high levels of DNA whenever practicable.

6.1.4. To maintain a separation in time and space between questioned and known samples:

6.1.4.1. At no time will questioned and known samples be simultaneously incubating in the same thermomixer or heat block.

6.1.4.2. At no time will questioned and known samples be simultaneously extracted in the same carrier.

6.2. Non-Differential Samples: This procedure may be performed in 200 µL, 500 µL, or 1000 µL lysis volumes.

6.2.1. **Casework ADV HE 200** (e.g., ¼ swab cuttings, reference samples, other small cuttings):

6.2.1.1. Preheat a thermomixer to 56°C.

6.2.1.2. Check the Buffer ATL to make sure it does not contain a white precipitate. If it does, place in the incubated orbital shaker for 30 minutes at 70°C with gentle agitation (approximately 100 rpm).

6.2.1.3. Gather samples to be processed in the batch along with the assigned reagent blank. **The order and labeling of initial tubes must be witnessed by a second trained individual.** All witness steps will be captured in the Batch Comments of the appropriate STACS documentation.

6.2.1.4. Add 180 µL Buffer ATL and 20 µL QIAGEN Proteinase K to each tube containing sample. Vortex well and quick spin down.

NOTE: The Buffer ATL and QIAGEN Proteinase K may be aliquoted as a master mix. If the master mix method is used, this aliquot expires at the end of the prepared date.

6.2.1.5. Incubate/shake tubes for 2 hours to overnight at 56°C and 900 rpm in a thermomixer. After digestion, vortex well and quick spin down the sample tubes.

6.2.1.6. Transfer the substrate to a filterless basket and spin at maximum speed for 2 minutes. Discard substrate and basket.

6.2.1.7. Transfer each lysate (approximately 200 µL) to a labeled, sterile 2.0 mL non-skirted tube (Sarstedt Inc., P/N 72.693).

NOTE: Do not transfer any solid material as this may clog the tips during automated DNA purification.

6.2.2. Continue with step 6.4: Procedure after Initial Lysis

6.2.3. **Casework ADV HE 500** (e.g., full swab cuttings, medium size cuttings or moderately soiled samples):

6.2.3.1. Preheat a thermomixer to 56°C.

6.2.3.2. Check the Buffer ATL to make sure it does not contain a white precipitate. If it does, place it in the incubated orbital shaker for 30 minutes at 70°C with gentle agitation (approximately 100 rpm).

6.2.3.3. Gather samples to be processed in the batch along with the assigned reagent blank. **The order and labeling of initial tubes must be witnessed by a second trained individual.** All witness steps will be captured in the Batch Comments of the appropriate STACS documentation.

6.2.3.4. Add 475 µL of Buffer ATL and 25 µL QIAGEN Proteinase K to each tube containing sample. Vortex well and quick spin down.

NOTE: The Buffer ATL and QIAGEN Proteinase K may be aliquoted as a master mix. If the master mix method is used, this aliquot expires at the end of the prepared date.

6.2.3.5. Incubate/shake tubes for 2 hours to overnight at 56°C and 900 rpm in a thermomixer. After digestion, vortex well and quick spin down the sample tubes.

6.2.3.6. Transfer the substrate to a filterless basket and spin at maximum speed for 2 minutes. Discard substrate and basket.

6.2.3.7. Transfer each lysate (approximately 500 µL) to a labeled, sterile 2.0 mL non-skirted tube (Sarstedt Inc., P/N 72.693).

NOTE: Do not transfer any solid material as this may clog the tips during automated DNA purification.

6.2.3.8. Continue with step 6.4: Procedure after Initial Lysis

6.2.4. **Casework ADV HE 1000** (e.g., 2 full swab cuttings, 1 cm x 1 cm cuttings, large size cuttings or heavily soiled samples):

6.2.4.1. Preheat a thermomixer to 56°C.

6.2.4.2. Check the Buffer ATL to make sure it does not contain a white precipitate. If it does, place it in the incubated orbital shaker for 30 minutes at 70°C with gentle agitation (approximately 100 rpm).

6.2.4.3. Gather samples to be processed in the batch along with the assigned reagent blank. **The order and labeling of initial tubes**

must be witnessed by a second trained individual. All witness steps will be captured in the Batch Comments of the appropriate STACS documentation.

6.2.4.4. Add 960 µL Buffer ATL and 40 µL QIAGEN Proteinase K. Vortex well and quick spin down.

NOTE: The Buffer ATL and QIAGEN Proteinase K may be aliquoted as a master mix. If the master mix method is used, this aliquot expires at the end of the prepared date.

6.2.4.5. Incubate/shake tubes for 2 hours to overnight at 56°C and 900 rpm in a thermomixer. After digestion, vortex well and quick spin down the sample tubes.

6.2.4.6. Transfer the substrate to a filterless basket and spin at maximum speed for 2 minutes. Discard substrate and basket.

6.2.4.7. Transfer each lysate (approximately 1000 µL) to a labeled, sterile 2.0 mL non-skirted tube (Sarstedt Inc., P/N 72.693).

NOTE: Do not transfer solid material as this could clog the tips during automated DNA purification.

6.2.4.8. Continue with step 6.4: Procedure after Initial Lysis

6.3. Differential Samples: This procedure is performed with an initial lysis volume of 500 µL to isolate material from non-sperm cells. Samples are typically the following: up to one full swab cutting, medium size cutting, or moderately soiled sample cutting. The non-sperm fraction lysates are processed using the Casework ADV HE 500 protocol. The remaining sperm cell fraction is then washed, lysed, and processed using the Casework ADV HE 200 protocol.

6.3.1. Preheat a thermomixer to 56°C.

6.3.2. Check the Buffer ATL to make sure it does not contain a white precipitate. If it does, place in the incubated orbital shaker for 30 minutes at 70°C with gentle agitation (approximately 100 rpm).

6.3.3. Gather samples to be processed in the batch along with the assigned reagent blank. **The order and labeling of initial tubes must be witnessed by a second trained individual.** All witness steps will be captured in the Batch Comments of the appropriate STACS documentation.

6.3.4. Add 475 µL Buffer ATL and 25 µL of QIAGEN Proteinase K. Vortex well and quick spin down.

NOTE: The Buffer ATL and QIAGEN Proteinase K may be aliquoted as a master mix. If the master mix method is used, this aliquot expires at the end of the prepared date.

6.3.5. Incubate/shake tubes for 1 hour at 56°C and 900 rpm in a thermomixer. After digestion, vortex well and quick spin down the sample tubes.

6.3.6. Transfer the substrate to a filterless basket and spin for 5 minutes at maximum speed. Discard substrate and basket.

6.3.7. Transfer all but approximately 30 µL of the supernatant to a new labeled, sterile 2.0 mL non-skirted tube (Sarstedt Inc., P/N 72.693) without disturbing the pellet. This is the non-sperm fraction that will proceed with step 6.4 Procedure after Lysis using the Casework ADV HE 500 protocol.

NOTE: Non-sperm fractions can be processed immediately on the QIAsymphony or can be stored at 4°C (for a maximum of overnight). If stored at 4°C, ensure samples come up to room temperature prior to further processing.

6.3.8. Re-suspend the pellet in 500 µL TE Buffer. Vortex and spin the samples in a microcentrifuge for 5 minutes at maximum velocity. Without disturbing the pellet, remove and discard all but approximately 30 µL of the supernatant.

6.3.9. Repeat step 6.3.8 for a total of 4 washes of the sperm pellet

NOTE: The wash step can be repeated an additional 1 to 5 times depending upon the nature of the sample.

6.3.10. OPTIONAL: Prepare a slide.

Re-suspend the pellet with the remaining 30 µL of supernatant by gently mixing the sample with a pipette. Remove approximately 4 µL of the sample and spot it on a glass microscope slide. Heat fix cells to the microscope slide following FBS07 – Microscopic Examination of Spermatozoa by Christmas Tree Stain (Document Control Number: 1577). The prepared slide(s) may also be stained and examined following FBS07.

6.3.11. OPTIONAL: Re-digest the sperm pellet.

If the slide is examined and intact epithelial cells are observed, an additional attempt to digest the non-sperm cells may be performed along with an additional wash. Follow these additional steps with the sperm fraction prior to proceeding to 6.3.12.

6.3.11.1. Add 475 µL of Buffer ATL to re-suspend the sperm pellet.

6.3.11.2. Add 25 µL of QIAGEN Proteinase K. Mix gently.

6.3.11.3. Incubate/shake each tube for 1 hour at 56°C and 900 rpm in a thermomixer.

6.3.11.4. After digestion, vortex well and spin at maximum velocity for 3 – 5 minutes. Remove and discard all but approximately 30 µL of the supernatant.

6.3.11.5. Re-suspend the pellet in 500 µL of TE Buffer and vortex. Spin the sample 3-5 minutes at maximum velocity. Remove and discard all but approximately 30 µL of the supernatant. Proceed to Step 6.3.12.

6.3.12. Preheat a thermomixer to 56°C.

6.3.13. Add 160 µL Buffer ATL, 20 µL QIAGEN Proteinase K, and 20 µL 1 M DTT to the pellet. Vortex well and quick spin down.

NOTE: The Buffer ATL, QIAGEN Proteinase K and 1 M DTT may be aliquoted as a master mix. If the master mix method is used, this aliquot expires at the end of the day.

6.3.14. Incubate/shake tubes for 1 hour to overnight at 56°C and 900 rpm in a thermomixer. After digestion, vortex well and quick spin down the sample tubes.

6.3.15. Transfer each lysate (approximately 200 µL) to a labeled, sterile 2.0 mL non-skirted tube (Sarstedt Inc., P/N 72.693).

6.3.16. Continue with step 6.4. Procedure after Initial Lysis.

6.4. Procedure after Initial Lysis

6.4.1. Things to do before starting:

6.4.1.1. Perform all maintenance (daily, weekly, monthly) before beginning any QIAsymphony SP instrument run. See FBQ46 – QIAsymphony Maintenance.

6.4.1.2. Check that Buffer QSL3 in the reagent cartridge does not contain a precipitate before using the reagent cartridge for the first time.

6.4.1.2.1. If there is a precipitate, remove the trough containing Buffer QSL3 from the reagent cartridge and incubate for 30 minutes at 37°C with occasional shaking (~100 rpm) to dissolve the precipitate. Once precipitate is dissolved place the QSL3 trough back into the reagent cartridge in the correct position.

6.4.1.3. If the reagent cartridge is already pierced, make sure that the troughs are sealed with the Reuse Seal Strips and incubate the whole reagent cartridge for 30 minutes at 37°C with occasional shaking (~100 rpm). Try to avoid vigorous shaking of the reagent cartridge otherwise foam may be generated, which can lead to liquid-level detection problems.

6.4.1.4. Use a vortex for approximately 3 minutes (or more if needed) to ensure that the magnetic particles in the trough are fully re-suspended. A previously opened trough may leak during vortexing, so it is recommended to cover it with a sterile paper towel to prevent spillage.

6.4.2. Turn on the QIAsymphony SP. Allow approximately 15 minutes.

6.4.3. Log in to the software.

6.4.4. Sample Preparation (per sample carrier):

6.4.4.1. Start the wizard located in the Sample Preparation screen.

6.4.4.2. Press Assay Control Sets and select from the following Investigator protocols:

CW 200 ADV HE V10
CW 500 ADV HE V10
CW 1000 ADV HE V10

6.4.4.3. Adjust the number of samples that will be processed on that sample carrier and press **Next** to continue.

6.4.5. Waste drawer:

6.4.5.1. Prepare the waste drawer by inserting the empty liquid waste container, tip chute, tip park station, empty unit boxes, and an empty tip disposal bag.

NOTE: Make sure the black rubber cover inside the waste drawer that is below the tip chute is on properly before closing the drawer.

NOTE: When the Wizard/load waste drawer screen is open only the waste drawer is unlocked.

6.4.5.2. Close the waste drawer after loading and Press **Next** to perform an inventory scan of the waste drawer.

6.4.6. Eluate drawer:

6.4.6.1. The **Wizard/Elution Slot/Configure Racks** screen will appear, and the eluate drawer can be opened.

6.4.6.2. Press the slot button in the touchscreen that a rack will be added to.

6.4.6.3. In the **Configure** tab, press **Rack ID** and enter an eluate rack ID. Alternatively, a bar code can be scanned if the eluate rack is bar code labeled. Select the elution rack type from the list: Tube 2.0mL, SAR#72.693 *T2.0 Screw.

6.4.6.3.1. If using the elution rack cooling in Elution Slot 1 make sure the snowflake button on the left of the slot is selected. The snowflake is gray when turned on.

NOTE: Use Elution slot 1 with the cooling adapter if eluates should be cooled.

6.4.6.4. Label cross-linked 2.0 mL Sarstedt 72.693 screw cap tubes with, at minimum, the sample name and tube number or associated bar code along the side of the tube and tube number on the cap of the tube. Load the eluate tubes into the corresponding locations in the eluate rack. Samples are eluted by the instrument vertically and then horizontally (A1, B1, C1, D1, then A2, B2, C2, D2, etc.).

The order and labeling of the sample and elution tubes as well as the loading of the tubes into the sample carrier(s) and eluate rack(s) must be witnessed by a second trained individual. See subsequent steps for loading of the tubes into the sample carrier(s).

6.4.6.5. Open the eluate drawer and place the eluate rack onto the elution slot selected with well A1 in the upper-left corner. Make sure the rack is held securely by the white pins. **Loading of the elution rack(s) on the QIAsymphony must be witnessed by a second trained individual.**

6.4.6.6. Repeat 6.4.6.2 through 6.4.6.5 if more elution racks need to be loaded.

6.4.6.7. Close the eluate drawer and press **Next**. An inventory scan of the eluate drawer will be performed.

6.4.7. Reagent and Consumables drawer: The **Wizard/Load Reagents** screen will appear summarizing the reagents and reagent containers needed.

6.4.7.1. Open the reagents and consumables drawer to load the required reagent cartridge(s) and consumables into the reagents and consumables drawer.

6.4.7.2. Place the reagent cartridge into the reagent cartridge holder. For first use remove the foil seal from the magnetic-particle trough before placing back into the reagent cartridge. After first use remove the cap covering the magnetic-particle trough. Remove the Reuse Seal Strips if using a partially used reagent cartridge.

6.4.7.3. Place the enzyme rack on the reagent cartridge holder then remove the lids from the tubes and place in the appropriate slot.

6.4.7.4. For first use place the piercing lid so the side with the opening fits against the magnetic-particle trough and the cutouts are around

the tubes of the enzyme rack. Gently push the piercing lid downward until it clicks in place.

NOTE: Ensure the buffer troughs fit correctly within the reagent cartridge otherwise liquid-level detection errors may occur.

NOTE: The piercing lid is sharp. Take care when placing it on to the reagent cartridge. Make sure to place the piercing lid onto the reagent cartridge in the correct orientation.

6.4.7.5. Slide the reagent cartridge along the slide rail into one of the reagent cartridge slots with the magnetic-particle trough oriented to the left.

6.4.7.6. Press the **Bottle ID** button to scan the bar code of the bottle of TopElute Fluid (TOPE) with the handheld bar code scanner or use the **Keyboard** screen to manually input the bottle bar code. Press **OK.**

NOTE: Ensure that the TopElute Fluid bottle is scanned, opened, and placed into the Reagents and Consumables drawer before starting otherwise the inventory scan must be repeated after scanning, opening, and placing the TopElute Fluid into the "Reagents and Consumables" drawer.

6.4.7.7. Place the bottle into the slot in front of the tip rack slots 1 and 2 with the cap removed. Press **Next** to continue.

6.4.7.8. The **Wizard/Load Consumables** screen appears after loading the reagents. Open the drawer and load the number of consumables displayed.

6.4.7.9. Close the drawer and the instrument will perform an inventory scan of the reagents and consumables drawer.

6.4.8. Sample drawer: The **Wizard/Sample Loading Summary** screen will appear summarizing the samples required for each sample rack.

6.4.8.1. Press **Next** and select the sample carrier type being used (tube carrier).

6.4.8.2. The **Wizard/Load Sample Tubes** screen will appear and indicates which samples should be loaded into which Sample slots.

6.4.8.3. Place the tubes in the appropriate insert in the tube carrier.

The order and labeling of the sample and elution tubes as well as the loading of the tubes into the sample carrier(s) and

eluate rack(s) must be witnessed by a second trained individual. See previous steps for loading of the tubes into the eluate rack(s).

6.4.8.4. Open the Sample drawer and gently slide the tube carrier into a free slot up to the stop line. Once the green LED starts to flash continue to push the tube carrier into the QIAsymphony SP instrument.

Loading of the carrier(s) on the QIAsymphony must be witnessed by a second trained individual.

6.4.8.5. Press **Next** to assign samples to a batch. The **Wizard/Batch X/Define Samples** screen will appear. Manually enter sample names, tube numbers, or select "Generate ID" for all samples. Press **Next**.

6.4.8.6. The **Wizard/Batch X/ Select Assay Control Sets** screen will appear. Samples not associated with a work list will be assigned an Assay Control Set.

6.4.8.7. Select samples that are to be processed with the same Assay Control Set. Then select the application in the **Applications/ACS** used with the selected samples (CW 200 ADV HE V10, CW 500 ADV HE V10, or CW 1000 ADV HE V10).

6.4.8.8. Press **Next** to get to the Wizard/Elution Slot & Volume screen.

6.4.8.9. Select the elution slot for the batch by pressing the corresponding slot button.

6.4.8.10. Select 40 µL for the elution volume and press **Queue** or **Finish** to finish the batch definition and close the wizard. If additional batched need to be defined, the Wizard continues with configuration of the next batch.

6.4.9. Press the **Run** button to start processing.

6.4.10. After the run is finished open the eluate drawer and retrieve the elution rack containing the purified DNA.

NOTE: Make sure to remove the elution rack from the inventory by selecting the elution slot and pressing the **Remove** button in the **Configure** tab. Once the elution rack is removed press **Yes** to continue.

6.4.11. Add a correctly labeled screw cap to each sample tube.

6.4.12. Store the samples frozen.

6.5. Post-Run Maintenance - After the completion of any QIAsymphony SP instrument run, perform the following steps:

6.5.1. Remove the TopElute fluid bottle from the instrument and place the cap back on the bottle.

6.5.2. Remove the used reagent cartridge(s) from the Reagents and Consumables drawer and discard appropriately if expired or empty. If the reagent cartridge(s) is only partially used, seal it with the provided Reuse Seal Strips and close the cRNA tubes with screw caps immediately after the end of the protocol run to avoid evaporation.

6.5.3. Remove sample tube carriers from the samples drawer and discard used sample tubes appropriately. Wipe the sample tube carriers as well as the eluate adapters with 70% ethanol followed by water.

6.5.4. Replace the tip disposal bag if full before starting the next run. Check the liquid waste container and discard appropriately, if needed, before starting the next run.

6.5.5. If it was the last run of the day, remove all remaining consumable plastic items from the instrument and discard appropriately or store for reuse later.

6.5.5.1. Save empty 8-rod cover and sample prep cartridge unit boxes to be used as waste unit boxes.

NOTE: The 8-rod cover unit box has a platform at the bottom that must be discarded prior to using as a waste unit box.

6.5.5.2. Close unit boxes filled with waste plasticware and discard appropriately.

6.5.5.3. Discard empty tip racks in the regular trash.

6.5.6. OPTIONAL: Perform a UV decontamination of the worktable.

6.5.7. Close all the workstation drawers.

6.5.8. Switch off the QIAsymphony SP.

7. Sampling

7.1. Not applicable

8. Calculations

8.1. Not applicable

9. Uncertainty of Measurement

9.1. Not applicable

10. Limitations

10.1. The quantity and quality of the DNA present within any biological material ultimately determines if a nuclear DNA isolation is successful.

10.2. Following quantitation, the remaining volume of a QIAsymphony extract is 38 µL. To allow for a replicate amplification (if needed), the minimum volume for an extract is 30 µL. If QIAsymphony extracts do not initially quantify with sufficient DNA to reach the amplification cutoff value, microconcentration is not expected to produce a noticeable improvement of the final DNA profile. If, however, indications of inhibition are observed during quantitation or detection, additional cleanup of the extract using the concentration procedure may be used to attempt to improve results.

10.3. The separation of non-sperm and sperm cell DNA into their respective fractions is not always complete. It is not unusual for sperm cell DNA to be observed in the non-sperm (or epithelial) cell fraction and vice versa. The number of intact cells recovered in a sample and their capacity to endure the abrasive conditions of the differential extraction method is dependent upon the quality of the biological material being tested and the environmental conditions to which it has been subjected. The detection of residual DNA within a given fraction does not prohibit the use of the DNA typing results from that fraction.

10.4. The presence of nuclear DNA in the sperm fraction of a differential extraction is not always a dependable method of determining whether semen or spermatozoa in a particular biological specimen is present. Many factors may influence this determination such as differing profiles in the sperm fraction and non-sperm fraction of the sample and the associated semen screening results (AP, p30, microscopic sperm identification).

10.5. Eluates should be capped and removed immediately following the end of a run to prevent evaporation. Samples should not be left on the instrument overnight.

10.6. Whenever possible, samples should be processed immediately following lysis steps. If circumstances occur where samples are not able to be immediately processed, lysates may be stored at 4°C for no more than 1-3 days.

11. Documentation

11.1. Applicable STACS documentation

12. References

12.1. QIAsymphony DNA Investigator Handbook (Document Control Number: 29522)

12.2. QIAGEN Validation Report – Developmental validation of the forensic QIAsymphony workflow (2013)

12.3. QIAsymphony SP/AS – Operating the QIAsymphony SP (Document Control Number: 29544)

12.4. QIAsymphony SP Protocol Sheet Casework_1000_ADV_HE_V10 protocol (Document Control Number: 29593)

12.5. QIAsymphony SP Protocol Sheet Casework_500_ADV_HE_V10 protocol (Document Control number: 29549)

12.6. QIAsymphony SP Protocol Sheet Casework_200_ADV_HE_V10 protocol (Document Control Number: 29547)

12.7. Microscopic Examination of Spermatozoa by Christmas Tree Stain (FBS07)

12.8. DC DFS Internal Validation: QIAsymphony SP Instrument using the QIAsymphony DNA Investigator Kit (4/13/2022)